**SCOPES OF THE PROJECT**

Current chemotherapy for the two major filarial diseases, onchocerciasis and lymphatic filariasis, targets the microfilarial stage of the parasites and temporarily sterilizes adult nematodes, therefore lengthy treatment regimens are required to cover the reproductive life-span of the long-lived adult worms. Programme success is constrained by the absence of drugs with macrofilaricidal activity and concerns of developing drug resistance in human parasites.

The main goal of this project is to identify both repurposed and novel molecules as potential new treatments for filarial infections, with a focus on macrofilaricides for onchocerciasis. However, drug discovery for this disease has to rely on surrogate parasites because the only viable host for O. volvulus is humans. DNDi has prioritised screening of selected compound libraries with a greater probability of yielding drug candidates. These include:

1. Indication sets (compounds which have progressed to preclinical or clinical research but failed to reach the market)
2. Chemical series from veterinary anti-infective research programs
3. Well annotated sets of compounds (i.e. bioavailable sets, compounds which have been through lead optimization, chemical series from anti-infective research programs etc.)

**SCREENING CASCADE**

The primary in vitro screens target Onchocerca species: O. gutturoso adult worms (5-day motility/MTT assay) and O. lienalis microfilariae, mf (5-day motility assay). Compounds with in vitro activity in the micromolar (µM) range are considered hits.

In vivo proof of principle is established in gerbils or mice infected with Litomosoides sigmodontis and is conducted on hits with good oral PK in the relevant host species. The dosing regimen is adjusted to reach plasma concentrations above EC50 for 24 hours. This model is regarded as a reasonable predictor of clinical efficacy.

**Summary**

- Several primary hits identified from screening a well-annotated compound collection against Onchocerca adult worms have demonstrated in vivo activity in a L. sigmodontis model.
- The combination of structure activity relationships and in vivo proof of principle identified three distinct chemical series of interest.
- These three series were identified from programs that targeted the human histamine H3, 5-hydroxytryptamine-6 (5-HT6) or sphingosine-1-phosphate-1 (S1P1) receptors.
- These novel anti-filarial scaffolds are currently being advanced in hit-to-lead studies.
- The screening cascade elaborated for this project captures both in vitro and in vivo activity of molecules using two surrogate species: O. gutturoso in vitro and L. sigmodontis in vivo.
- This cascade may be useful in hit to lead and lead optimization programs as well.
- The lack of activity of these compound series against mf indicates that paradigms utilizing low throughput adult worm assays as primary screens can successfully identify novel macrofilaricide chemotypes.

**Litomosoides sigmodontis in vivo filariasis model**

- **L. sigmodontis Life Cycle**
  - Litomosoides sigmodontis is transmitted by a small bite, *Onchophyrous bacoti*.
  - The parasitized mite transmits third-stage larvae during a blood meal.
  - The larvae then migrate from the skin, through the lymphatics, to the thoracic (pleural) cavity where they mature and breed.
  - The adult female worms release their microfilariae in the pleural cavity from which they make their way to the blood, ready to be taken up by a mite feeding on the skin.

Onchocerca gutturoso adult worm in vitro motility/MTT assay

- Biochemical evaluation of worm viability using MTT/formazan colorimetry
- Natural infection in vivo and infection in vitro

Efficacy Model

When the treatment begins, adult worms are not fully mature and do not produce microfilariae (mf).

1. BALB/c are exposed to infected mites (*Onchophyrous bacoti*), for 24 h.
2. After inoculation with infective larvae (L3), migration occurs via the lymphatic system to the pleural cavity by Days 2-6 post-infection (p.i.)
3. Between Days 8-12 p.i., larvae molt into L4 stage, then mature to adults by Day 30 p.i.
4. At Day 30 p.i., animals are randomized, allocated in groups of 6 animals and treated.
5. At Days 60-90 p.i., microfilariae are produced and released in blood

**Screening of the Abbvie Bioavailability Set**

A prioritized subset of the Abbvie compound collection was assembled based on favorable oral bioavailability and/or low clearance in previous animal pharmacokinetic studies (AbbVie Bioavailability Set).

The Bioavailability Set was clustered based on structure to 760 representative compounds, which were screening against adult O. gutturoso adult worms.

Cross screening of the adult screening hits against O. lienalis microfilariae displayed no overlap in vitro activity, suggesting that the adult worm screen had identified primarily specific macrofilaricides. Screening of structural analogs around the original hits, facilitated by chemoinformatic analysis, led to a dramatically improved second-round hit rate and, in some cases, identification of compounds with improved in vitro potency.

Three compounds demonstrated in vivo activity against Litomosoides sigmodontis in mice.

**Screening Results**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Adult Females</th>
<th>Adult Males</th>
<th>Microfilariae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flubendazole</td>
<td>6 mg/kg</td>
<td>5.8 (3.5)</td>
<td>5 (3)</td>
<td>15 (8)</td>
</tr>
<tr>
<td>A-697365</td>
<td>200 mg/kg</td>
<td>10 (14)</td>
<td>12 (14)</td>
<td>0</td>
</tr>
<tr>
<td>A-697365</td>
<td>100 mg/kg</td>
<td>2.0 (2.3)</td>
<td>1.2 (1.4)</td>
<td>0</td>
</tr>
<tr>
<td>A-106844</td>
<td>40 mg/kg</td>
<td>4.6 (2.4)</td>
<td>2.2 (2.3)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Efficacy Results in a Murine L. sigmodontis Model**

- **Recovered adult worms**
  - 75 days post infection
- **Microfilariae in blood**
  - Concentration over time of microfilariae

**Results from Analog Screening**

- **Compound**
- **Human Target**
- **Number of Analogs Tested**
- **Score 3 Hits**
- **Score 2 Hits**
- **Improved Potency**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human Target</th>
<th>Number of Analogs Tested</th>
<th>Score 3 Hits</th>
<th>Score 2 Hits</th>
<th>Improved Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-697365</td>
<td>H3</td>
<td>85</td>
<td>18 (23%)</td>
<td>20 (34%)</td>
<td>A-641227 EC50 = 3.1 µM</td>
</tr>
<tr>
<td>A-697365</td>
<td>S1P1</td>
<td>147</td>
<td>30 (10%)</td>
<td>42 (28%)</td>
<td>A-1023700 EC50 = 0.24 µM</td>
</tr>
<tr>
<td>A-106844</td>
<td>S1P1</td>
<td>49</td>
<td>20 (43%)</td>
<td>8 (16%)</td>
<td>A-1023701 EC50 = 0.77 µM</td>
</tr>
</tbody>
</table>

**Footnotes**

- NPMRI: National Parasitological Research Institute, India
- ICN: International Centre for Norbeck Research, Ghana
- DNDi: Drug for Neglected Diseases initiative, Switzerland